

# Plasmid Midiprep Kit (Endotoxin Free)



# EBL Plasmid Midiprep Kit(Endotoxin Free)

**Storage:** Stored at room temperature (15-25°C).  
**Format:** spin column **For research use only**  
**Sample:** about 50-300ml cultured cell

## Introduction:

The EBL Endotoxin-Free Midiprep Kit is designed for plasmid DNA purification and endotoxin removal. It utilizes pre-packed anion-exchange resin midiprep columns to purify plasmid DNA from 50–300 mL of cultured bacterial cells. To prevent common handling errors, the kit includes Eblue Lysis Buffer, an optional color indicator that ensures efficient cell lysis and neutralization. This endotoxin-free kit employs a modified alkaline lysis method alongside RNase treatment to yield a clear cell lysate with minimal genomic DNA and RNA contamination. Through an efficient gravity-flow procedure, plasmid DNA from the crude lysate binds to the column, while contaminants are eliminated using a wash buffer. The purified plasmid DNA is then eluted with a high-salt buffer and desalted via isopropanol precipitation. The entire procedure can be completed in 80 minutes without the need for ultracentrifugation or toxic reagents. The resulting highly purified plasmid DNA is ideal for demanding downstream applications, including transfection, sequencing, ligation, PCR, in vitro transcription, microinjection, restriction enzyme digestion, and gene gun delivery.

## About the kits:

|                    |             |
|--------------------|-------------|
| Cat. No.           | MPD-02025EF |
| 400PLUS Columns    | 25 pcs      |
| Buffer IP1         | 110 ml      |
| Buffer IP2         | 110 ml      |
| Buffer IP3         | 110 ml      |
| Buffer BEF         | 40 ml       |
| Buffer BEQ         | 130ml       |
| Buffer WB          | 125ml+260ml |
| Buffer HEB         | 220ml       |
| RNase A (50mg/ ml) | 200 µl      |
| Eblue Lysis buffer | 1.5 ml      |

**Materials required but not provided:** 75% ethanol, isopropanol, TE or DI water

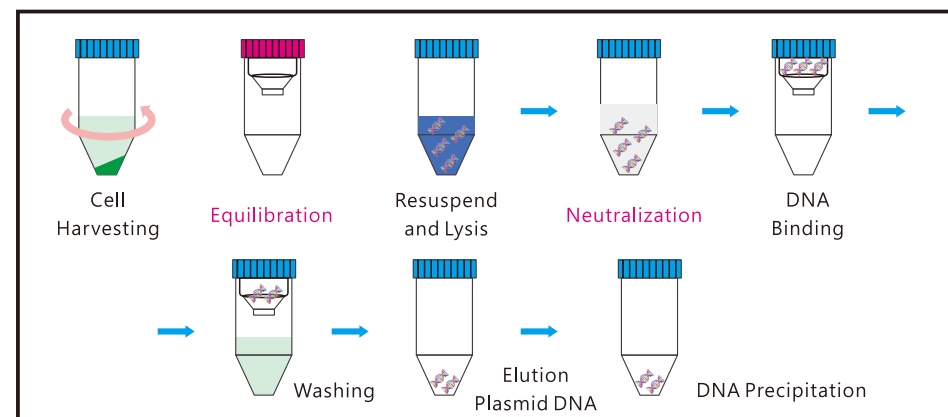
### NOTE:

1. Add the provided RNase A solution to Buffer IP1 and mix well then store at 2–8°C for up to 6 months.
2. If necessary, re-dissolve any precipitate by warming to 37°C.
3. Buffer IP 2, 3 and Buffers WB contain irritants. Wear gloves when handling these buffers.

## Related product and Ordering information:

| CAT NO:   | Product                                    | Quantity |
|-----------|--------------------------------------------|----------|
| MGK-T0100 | EBL Genomic DNA Isolation Kit (Tissue)     | 100 rxn. |
| MGK-T0300 | EBL Genomic DNA Isolation Kit (Tissue)     | 300 rxn. |
| MGK-C0100 | EBL Genomic DNA Isolation Kit(Cell/ Blood) | 100 rxn. |
| MGK-C0300 | EBL Genomic DNA Isolation Kit(Cell/ Blood) | 300 rxn. |
| MGK-P0100 | EBL Genomic DNA Isolation Kit(Plant)       | 100 rxn. |
| MGK-P0300 | EBL Genomic DNA Isolation Kit(Plant)       | 300 rxn. |
| MPD-01300 | EBL Plasmid Miniprep DNA Kit               | 300 rxn. |
| MPD-02025 | EBL 200PLUS Plasmid Midiprep DNA Kit       | 25 rxn   |
| MPD-03020 | EBL 800PLUS Plasmid Maxiprep DNA Kit       | 20 rxn   |
| FBRE100   | Blood/ cell Total RNA Isolation kit        | 100 rxn. |
| FBRT100   | Tissue Total RNA Isolation kit             | 100 rxn. |
| FBRP      | Plant Total RNA Isolation kit              | 100 rxn. |

## Procedure:



## Protocol:

### Cultured Cells Harvesting

1. Transfer 50–300 ml bacterial culture to a centrifuge tube. Depending on the plasmid copy number, approximately 50–150 mL of cell culture (approx. 0.75 g wet pellet weight) should be used for high-copy plasmids. If Low-copy plasmids, use approximately 100–300 mL of bacterial culture (yielding about 1.2 g wet pellet weight).
2. Centrifuge at 6,000 x g for 10 minute and discard the supernatant.

### Equilibration

3. While the sample is centrifuging, prepare a 400PLUS Column by placing it into a new 50 mL centrifuge tube. Add 5 mL of BEQ Buffer to equilibrate the column, allowing it to empty completely via gravity flow. Discard the flow-through, return the column to the 50 mL centrifuge tube, and set it aside for DNA binding (Step 7).

### Resuspend

4. Resuspend bacterial cells pellet in 4 ml of Buffer IP1 and 40µl Eblue Lysis buffer.
- # Add the provided RNase A solution to Buffer IP1 and mix well then store at 2–8°C.

### Lysis

5. Add 4 ml of Buffer IP2 and mix thoroughly by inverting the tube 10 times (Do not vortex) and then stand at room temperature for 2 minutes or until the lysate is homologous.
- NOTE:** After adding Buffer IP2, any precipitates should dissolve completely and the suspension will turn blue. If the suspension contains colorless regions or brownish cell clumps, continue mixing until it becomes a uniform blue color.

### Neutralization

6. Add 4 ml of Buffer IP3 and mix immediately and thoroughly by inverting the tube 10 times (Do not vortex). Centrifuge at 6,000xg for 20 minutes.

## Endotoxin Removal

**NOTE:** Invert the Buffer BEF bottle 3–5 times immediately prior to use.

Transfer the supernatant to a clean 50 mL centrifuge tube. Add 1.2 mL of Buffer BEF, then mix by inverting 5–10 times. **Incubate on ice for 30 minutes.**

**NOTE:** Following the addition of Buffer BEF, the mixture will become cloudy.

## DNA Binding

7. Place a 400PLUS Column in a 50 ml centrifuge tube.

8. Transfer the supernatant to the equilibrated 400PLUS Column. Allow the column to empty completely by gravity flow. Discard the flow-through, then place the 400PLUS Column back into the 50 mL centrifuge tube.

## Wash

9. Add 15 ml of Buffer WB into the 400PLUS Column and allow it to empty completely via gravity flow. Discard the flow-through, then place the column to a new 50 mL centrifuge tube.

## Elution

10. Add 8 ml Buffer HEB to elute the plasmid DNA by gravity flow.

## DNA Precipitation

11. Add 6 mL (0.75x volumes) of isopropanol to the eluted DNA from Step 10. Mix the tube completely by inverting, then centrifuge at 23,000 x g for 20 minutes at 4°C.

12. Carefully remove the supernatant, then wash the DNA pellet with 5 mL of 75% ethanol. Centrifuge at 23,000 x g for 5 minutes at 4°C. Carefully remove the supernatant, then air-dry the DNA pellet for 10 minutes.

13. Once the DNA pellet is dry, add 500 µL–2 mL (or a suitable volume) of TE Buffer<sup>1</sup> or water<sup>2</sup>, then place the tube in a 60°C water bath for 5–10 minutes to dissolve the DNA pellet.

**NOTE:** Following both centrifugation steps, extra caution is needed when removing the supernatant to avoid disturbing the DNA pellet.

<sup>1</sup> Using TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) is beneficial, as EDTA preserves DNA for long-term storage. However, EDTA will affect PCR and other sensitive downstream applications.

<sup>2</sup> If using water, ensure the pH is  $\geq 8.0$ . ddH<sub>2</sub>O should be fresh, as ambient CO<sub>2</sub> can quickly cause acidification.

## Troubleshooting

### Problem

### Comments and suggestions

#### Presence of RNA

> RNA contamination

Ensured RNase A is added before using Buffer IP1.

#### Plasmid bands was smeared on agarose gel

> plasmid DNA degradation

Keep plasmid preparations on ice or frozen in order to avoid plasmid DNA degradation.

#### Presence of genomic DNA

> Genomic DNA contamination

1. Do not overgrow bacterial cultures.
2. Do not incubate more than 5min after added Buffer IP2.

#### Low yields of DNA

> Low plasmid copy number

1. Increase the culture volume.
2. Change the culture medium.

> Nuclease contamination

1. Check Buffers for nuclease contamination and replace if necessary.
2. Use new 50 ml centrifuge tube and wear gloves.

> Column overloaded

Decrease the loading volume or lower the culture density.

> SDS in the Buffer IP2 precipitated

The SDS in Buffer IP2 may precipitate with storage. If this happens, incubate the Buffer IP2 at 30~40°C for 5 min and mix well.

> Incorrect elution conditions

Ensure that Buffer HEB is added into the center of the 400PLUS Column.

> Plasmid lost in the host E. coli

Prepare and use fresh culture.

#### Inhibition of downstream enzymatic reactions

> TE buffer used for DNA elution.

Use the ethanol to precipitate the DNA, or repurify the DNA fragments and elute with the nuclease-free water.

> Presence of residual ethanol in plasmid.

To ensure the complete removal of ethanol, you can perform an additional brief centrifugation step or extend the air-drying time.